PERMEABILITY OF THE POSTERIOR LENS CAPSULE IN VITRO AND IN VIVO

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1986

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by

Betty Rae Klein

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ABSTRACT

Permeability of the Posterior Lens Capsule

In Vitro and In Vivo

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Extracapsular cataract surgery has resulted in increasing numbers of aphakic eyes with the posterior lens capsule present. According to recent clinical and experimental observations, an intact posterior lens capsule may increase the life expectancy of the aphakic eye by decreasing subsequent vitreal and retinal disease.

We measured apparent permeability coefficients of rabbit and human posterior lens capsules in an *in vitro* diffusion chamber and by perfusion of living phakic and aphakic rabbit eyes. The mean permeability coefficients of isolated rabbit posterior lens capsules to fluorescein sodium (MW = 376) and three sizes of fluorescein labelled dextran (MW = 4000, 18,900, and 40,000) *in vitro* were $5.41 .728 \times 10^{-16}$, $6.35 \pm 4.41 \times 10^{-16}$, $4.32 \pm 1.24 \times 10^{-16}$, and $7.61 \pm 1.58 \times 10^{-17}$ mole/sec.dyne, respectively. The permeability coefficients of isolated human posterior lens capsules for fluorescein sodium, the 18,900 molecular weight dextran, and the 40,000 molecular weight dextran were $3.59 \pm 1.80 \times 10^{-16}$, 4.47×10^{-16}, and $1.65 \pm .165 \times 10^{-17}$

mole/sec.dyne, respectively. Greater variability was observed in the permeability of human capsules compared to albino rabbit capsules. The relationship between solute flux and concentration was linear, consistent with the model of the capsule as an inert semipermeable membrane.

The movement of fluorescein from the anterior chamber to the vitreous in intracamerally perfused living phakic eyes and aphakic eyes (with and without posterior capsulotomies) was investigated in rabbits. The permeability coefficient for living aphakic eyes with an intact posterior lens capsule ($1.60 \pm 1.02 \times 10^{-15}$ mole/sec.dyne) was three times greater than for isolated posterior lens capsules in vitro ($5.41 \pm .728 \times 10^{-16}$ mole/sec.dyne). For living aphakic eyes with posterior capsulotomy, the permeability coefficient ($7.30 \pm 6.65 \times 10^{-14}$ mole/sec.dyne) was 135 times greater than for isolated posterior lens capsules and 46 times greater than in eyes with intact posterior capsules ($1.60 \pm 1.02 \times 10^{-15}$ mol/sec.dyne).

BACKGROUND

Surgical extraction is the only treatment presently available for cataracts. Intracapsular extraction involves removing the lens in toto along with its elastic lens capsule. Extracapsular cataract extraction consists of incision of the anterior lens capsule, expression of the lens nucleus through the corneal wound, and aspiration of the lens cortex from the posterior chamber[1]. The posterior lens capsule is thus preserved in extracapsular but not intracapsular cataract extraction. Cataract extraction can sometimes be followed by a variety of vitreoretinal complications including retinal breaks, detachments, and cystoid macular edema[2]. Preliminary studies indicate that aphakic eyes with an intact posterior lens capsule have a lower incidence of posterior vitreous detachment[2] and angiographically confirmed postoperative aphakic cystoid macular edema (ACME) than eyes having had intracapsular lens extraction[3,4]. When primary or secondary capsulotomy is performed either by surgical discission or Neodymium:YAG laser, the incidence of posterior vitreous detachment[2] and of ACME again become increased[5,6]. The presence of an intact posterior lens capsule may reduce the frequency of post-operative retinal pathology and thus may help preserve a good visual result.

The Posterior Lens Capsule and Cystoid Macular Edema

Traction is a likely causative factor in the retinal tears and detachments typically occurring after cataract surgery[2]. However, there is no conclusive evidence for the cause of aphakic cystoid macular edema[7]. An association between ACME and anterior segment inflammation[8-10] and elevated aqueous prostaglandin levels[11] has been observed. The changes observed in

ACME could conceivably represent the actions of prostaglandin and/or other inflammatory substances diffusing posteriorly from the anterior segment after cataract surgery [12-15]. An intact posterior lens capsule may reduce the eye's susceptibility to cystoid macular edema by inhibiting movement of these substances from the aqueous posteriorly to the retina [12,14]. The relative contributions of the lens, posterior lens capsule, and surrounding zonule in inhibiting posterior diffusion of prostaglandins are not known.

Cystoid macular edema is characterized by dilation of the perimacular capillaries and leakage of protein-rich fluid into the loosely structured and tangentially oriented outer plexiform and inner nuclear retinal layers [16-19]. An altered foveal reflex is noted on direct and indirect ophthalmoscopy while slit-lamp biomicroscopy discloses macular thickening and the formation of microcystoid spaces [18]. Fluorescein angiography in these eyes typically shows collection of fluorescein in these spaces in a petaloid or rosette configuration [18,20].

Histologic changes in cystoid macular edema are suggestive of a prostaglandin induced phenomenon [21]. In the eye, prostaglandins cause leukotaxis, vasodilatation, and increased vessel permeability [22,23]. Aphakic cystoid macular edema shows disruption of the blood-retinal barrier [18] with leakage of serous exudate from intraretinal capillaries in the foveal region [16].

Specialized cells regulate the volume and composition of the small retinal extracellular spaces [24-26]. The retinal vascular endothelium and retinal pigment epithelium have tight intercellular junctional complexes (zonula occludens) separating intravascular and extracellular fluid media [24,25]. In avascular regions of the retina the Muller cell is important

in distributing nutrients, removing waste products, and transporting fluid[18]. The foveal avascular zone has fewer Muller cells and higher local tissue compliance than other parts of the retina[16,18,25,27]. This predisposes the macular region to disturbances of extracellular fluid volume and solute composition[16]. Injury to the blood-retinal barrier and/or Muller cell may result from ischemia[16,28,29], endogenous[28], or exogenous agents[30], vitreous traction[31,32] or a combination of these factors.

Cystoid macular edema occurs with a variety of ocular conditions in addition to cataract surgery[9]. These include retinal surgery, uveitis, ocular tumors, diabetic retinopathy, senile macular degeneration, atherosclerotic vascular disease[8], retinitis pigmentosa[10], topical epinephrine in aphakic eyes[33], nicotinic acid toxicity, radiation retinopathy, surface wrinkling retinopathy, choroidal lesions with submacular retinal pigment epithelial defects, and idiopathic forms[10]. The association of cystoid macular edema with a wide variety of conditions has suggested to some that it represents a common macular response to diverse ocular diseases[18]. Others contend that there are different varieties of cystoid macular edema depending on the cause[34]. Either way, there may be a common pathway for damage[13].

An association between post-cataract extraction cystoid macular edema and both clinical[12] and histologic[19] retinal phlebitis, cyclitis and vitritis has been recognized. ACME is also more common in eyes with anterior segment complications of cataract extraction such as iris or vitreous incarceration in the wound, retained lens cortex or uveitis[8,10] which produce mild inflammation of the ciliary body and vitreous[8-10]. If this anterior segment inflammation represents a vascular response to biotoxic

chemicals elaborated following ocular trauma[12-15], these same substances may be involved in the pathogenesis of aphakic cystoid macular edema. Inflammatory mediators transmitted by the vitreous may be more important in stimulating retinal vessels to leak than direct injury to the vessels themselves[8].

A recent clinical study investigated the role of ultraviolet light exposure following cataract extraction in ACME[30]. Placement of an ultraviolet absorbing chromophore in a posterior chamber intraocular lens reduced the incidence of postoperative cystoid macular edema (documented by fluorescein angiography) by 50 percent[30]. The mechanism underlying this phenomenon is not known. The release of prostaglandin in response to free radical production by the retinal pigment epithelium has been postulated[3,17].

Miyake found elevated aqueous levels of prostaglandin E and F alpha in eyes after intracapsular or extracapsular lens extraction[35,2]. These elevations were suppressed by pretreatment with topical indomethacin[35]. Elevated aqueous levels of prostaglandins E and F alpha detected in eyes with persistent cystoid macular edema secondary to vitreous incarceration in the surgical wound were reduced following vitrectomy[2,11]. Clinically, inhibitors of prostaglandin synthesis have been effective in the prevention of aphakic cystoid macular edema[7,15,36].

Although the above studies do not rule out the concurrent involvement of other endogenous substances produced in the eye (i.e. leukotriene, substance P, and ATP), they support the role of aqueous prostaglandins in the posterior segment pathology of ACME.

Efforts to induce prototypic cystoid macular edema in laboratory animals have been unsuccessful [37-39]. The association of CME with aphakia in humans most likely represents the combination of predisposing factors common to this setting [39]. Vascular fragility and inadequate tissue reparation may increase the susceptibility of older patients to chronic cystoid maculopathy [37,39]. Mechanical factors such as traction secondary to forward movement of the vitreous and loss of hyaluronic acid from the vitreous leading to liquefaction after intracapsular lensectomy may also play a role [20,31,32]. Firm attachments of vitreous collagen to the retina are present at the macula, disc margin, posterior surface of the posterior lens capsule, and pars plana [40]. These may predispose the macula to damage when the vitreous undergoes central liquefaction [2,20,32]. Although the inability to produce an animal model does not exclude the possibility of a mediator-receptor interaction in ACME [13], it implies a variety of factors interacting in the clnical setting in which this disease occurs [39].

The Posterior Lens Capsule and Iris Neovascularization

Diabetic patients with retinal neovascularization have an increased incidence of developing neovascularization of the iris (rubeosis iridis) following lensectomy with vitrectomy [41,42]. Removal of the lens and vitreous may predispose patients to this condition by permitting anterior diffusion of a vasoproliferative factor liberated from hypoxic retina [43,41]. This interpretation is supported by studies showing a similar increase in iris rubeosis in eyes which are aphakic before undergoing vitrectomy and no predisposition of eyes with cataracts to this disease [43]. Although an effort is now made to preserve clear lenses in eyes undergoing vitrectomy for complications of

diabetic retinopathy, the barrier function of the lens-zonular diaphragm to potential angiogenic factors has not been experimentally determined.

The retina may supply factors which stimulate the development of intraocular neovascularization[44]. Extracts from human and bovine retina have produced vasoproliferation on chick chorioallantoic membrane[45]. Vitreous extracts have been shown to inhibit this neovascularization[46]. Recently, retinal pigment epithelial cells in culture have been found to produce an inhibitor of neovascularization[47]. The growth of retinal vessels may depend on a balance of retina and RPE derived stimulators and vitreous derived inhibitors[44].

A vasoproliferative factor liberated from hypoxic retina has been postulated[41]. New blood vessel development is associated with vascular endothelial cell migration and increased incorporation of thymidine[48]. A potent stimulator of endothelial cell thymidine uptake has been partially purified from bovine retina. This substance, an anion of greater than 50,000 dalton molecular weight[49], also has potent chemoattractant activity for vascular endothelial cells in vitro[50]. Putting these experimental findings together with clinical observations in patients with rubeosis iridis, a model is proposed whereby a retina derived substance diffuses anteriorly to stimulate vascular endothelial growth on the iris[41]. The increased incidence of rubeosis iridis in vitrectomized aphakic eyes may represent unopposed action of vasoproliferative factor(s) following removal of vitreous derived inhibitor(s).

Barrier Function of the Posterior Lens Capsule

The posterior lens capsule may inhibit the transport of some aqueous solutes from the anterior and posterior chambers into the vitreous cavity[12,14] and of vitreous components into the anterior segment[51]. Clinical and experimental data suggest that the posterior lens capsule inhibits anterior displacement of the vitreous body[2] and anterior diffusion of hyaluronic acid, a vitreous structural molecule[51]. In addition, an intact posterior lens capsule has been shown to decrease the extension of bacterial infection from the anterior chamber into the vitreous cavity[52]. Whether or not an intact posterior lens capsule would reduce the susceptibility of eyes with retinal neovascularization to iris rubeosis is not known.

The potential barrier functions of the posterior lens capsule, capsular-zonular diaphragm, lens-zonular diaphragm, and anterior hyaloid face are questions with important clinical implications. The access of aqueous substances to the retina and of vitreous substances to the anterior ocular structures in phakic and aphakic eyes has not been well studied. Understanding the permeability characteristics of these structures may aid in evaluating the above models for ACME[12,14] and rubeosis iridis[41-43].

MATERIALS AND METHODS

In Vitro Experiments

Paired plexiglass chambers were used for *in vitro* testing of posterior lens capsule permeability (Figure 1). The chambers were horizontally joined by four metal prongs which provided consistent, stable alignment. Two flat plexiglass discs (25 mm diameter, 5.0 mm thickness), each with a central 5.0 mm round hole, fit into the opposing surfaces of each chamber over a flat rubber washer (Figure 2). The excised lens capsule was supported between two 5.0 mm diameter rubber-o-rings fit into a shoulder surrounding the central hole in each plexiglass disc. When the apparatus was assembled, the chambers communicated via a .071 cm^2 aperture with a watertight seal. The integrity of this seal was tested using Parafilm between the rubber-o-rings, a fluorescein solution in one chamber, and distilled water in the opposite chamber. After 24 hours, there was no measurable fluorescence in the opposite chamber. An aquarium pump supplied a continuous stream of air into a narrow tube angled downward to each chamber [Figure 1 (arrow)] and provided constant stirring of solutions. Test solutions were added and samples removed via a vertical, cylindrical column (18 mm diameter, 25 mm height) communicating with each chamber. Evaporation was minimized by sealing the columns with Parafilm pierced by a 27 gauge needle for air escape.

Twenty-three rabbit and nine human eyes were opened by posterior incision of the sclera (Figure 3). The zonular segments were disrupted using forceps (Figure 4). A lens loop was placed beneath the anterior surface of

the lens and the lens removed (Figure 5). A 360-degree equatorial incision was made in the lens capsule using a Vannas scissor (Figure 6). A rubber-o-ring was placed on the surface of the posterior lens capsule and cut edges were pushed up around its outer circumference (Figure 7). Forceps were used to lift the ring and adherent capsule, and residual cortex was removed by irrigation with distilled water. The rubber-o-ring was placed, capsule side down, on the rubber-o-ring of the opposite chamber (Figures 8 and 9). Chambers were assembled with each rubber-o-ring in its corresponding depression (Figure 10) and the capsule supported across the aperture between chambers. A tight fit was maintained with the chambers in horizontal position by metal brackets with tightening screws (Figure 11).

A test solution containing either fluorescein or one of the higher molecular weight fluorescein-labelled dextrans was added to the chamber contacting the anterior surface of the posterior lens capsule. The opposite chamber was filled with an equal amount of distilled water. Broken capsules were readily recognized by rapid mixing of the two solutions and were discarded. At the experiment's start, a 1.0 ml sample from each side of the capsule was removed to a clear plastic cuvette and fluorescence measured in an Amico-Bowman spectrophotofluorometer. Each sample was then returned to its chamber. At recorded intervals of 1 to 2 hours, samples were measured and returned in a similar manner. The experiment was performed with solutions at room temperature (293) degrees Kelvin).

For fluorescein, and for each of the fluorescein-labelled dextrans, a standard curve was constructed relating fluorescence to known solute concentration (Figure 12). The relationship was linear for the range of test

Figure 1

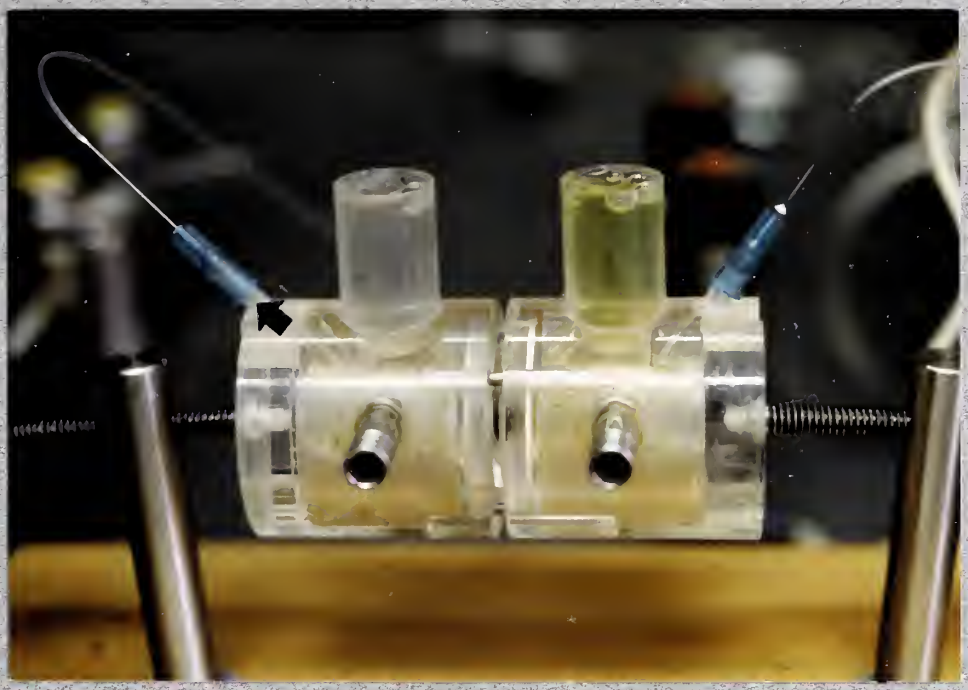


Figure 2

Figure 3

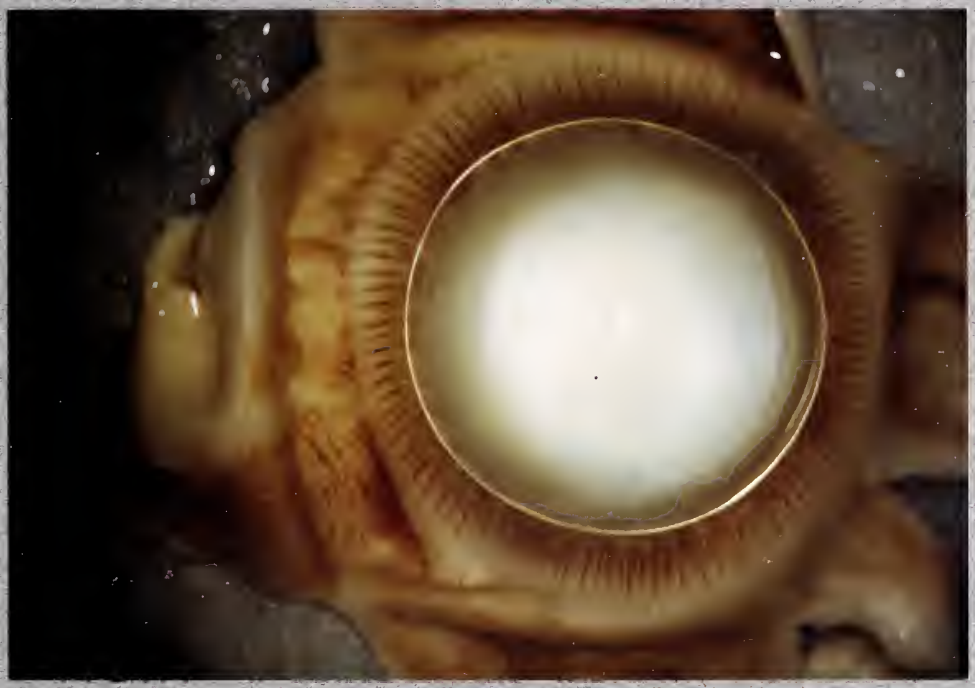


Figure 4

Figure 5

Figure 6

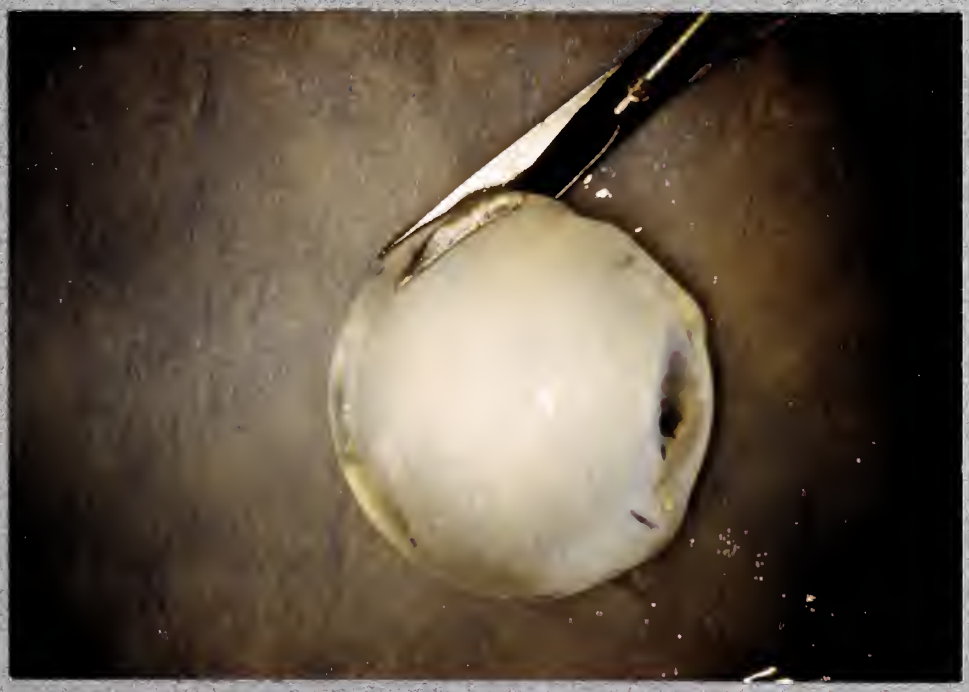


Figure 7

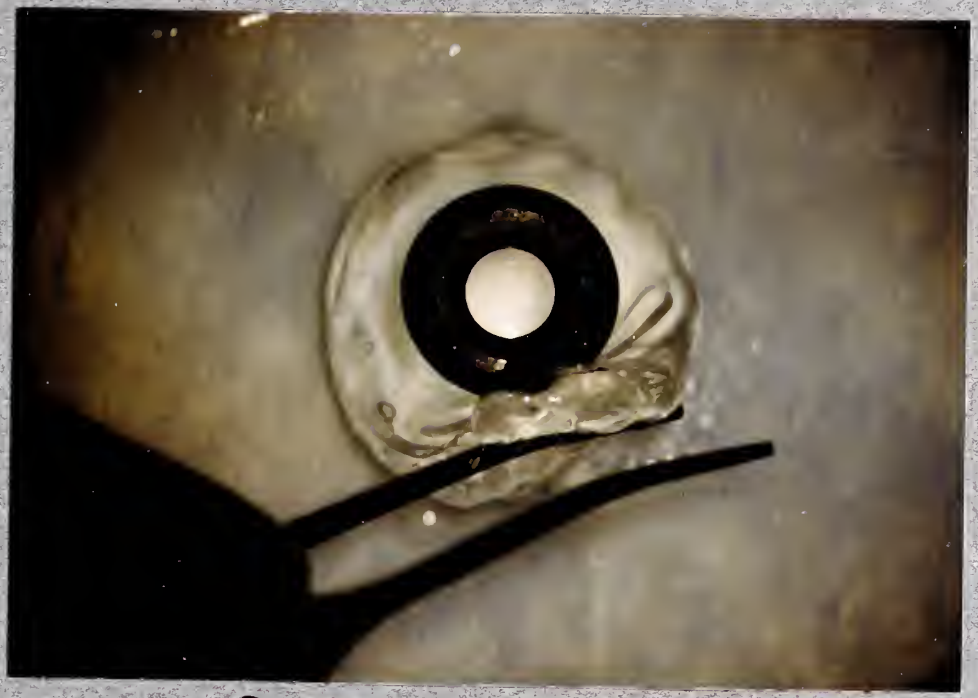


Figure 8

Figure 9

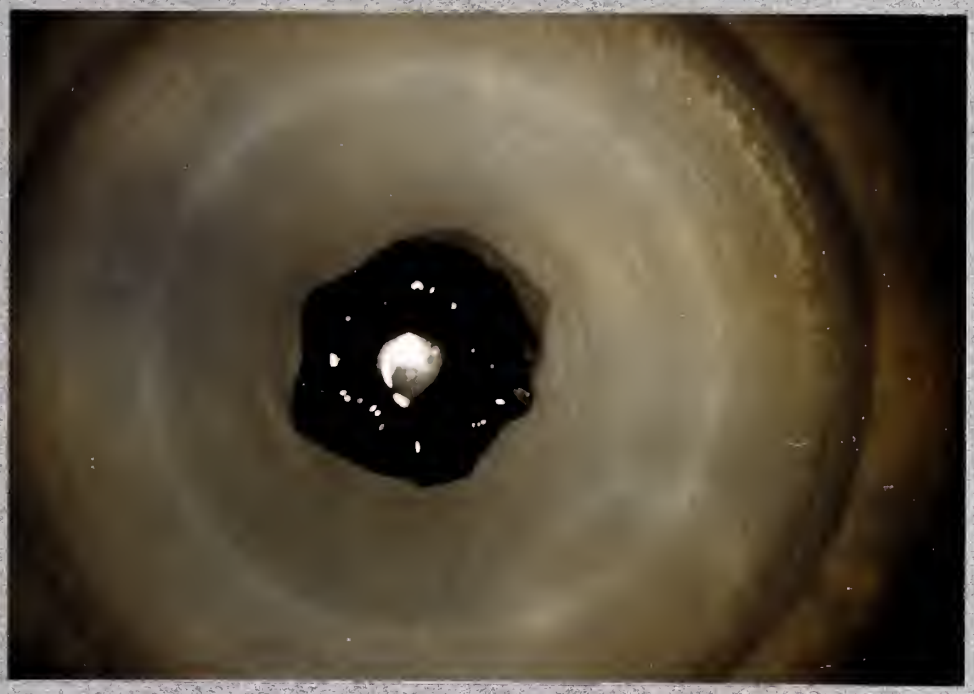


Figure 10

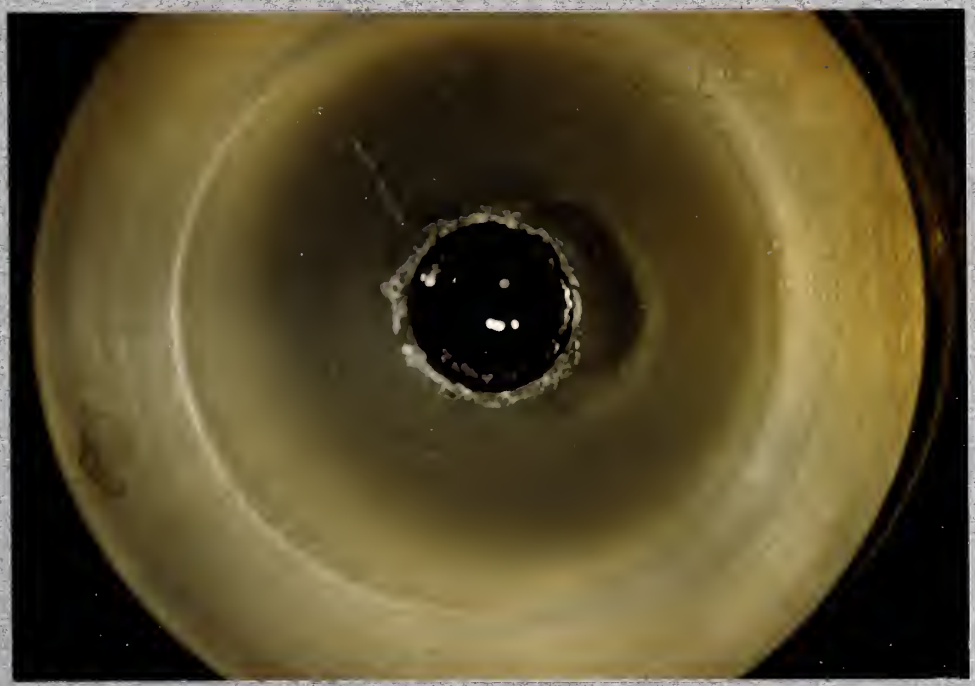


Figure II

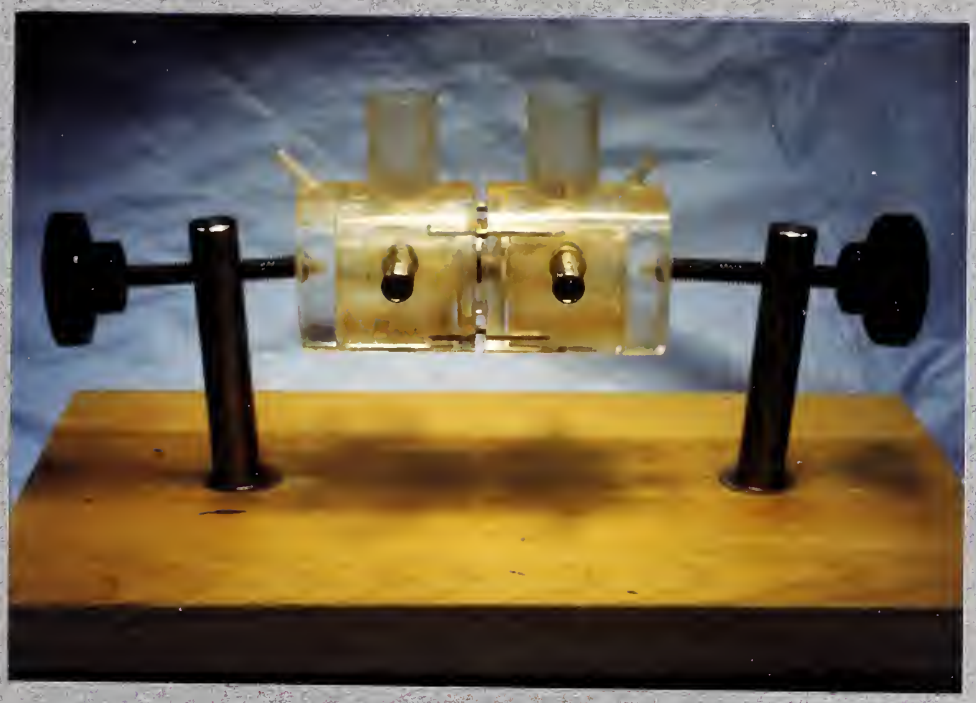
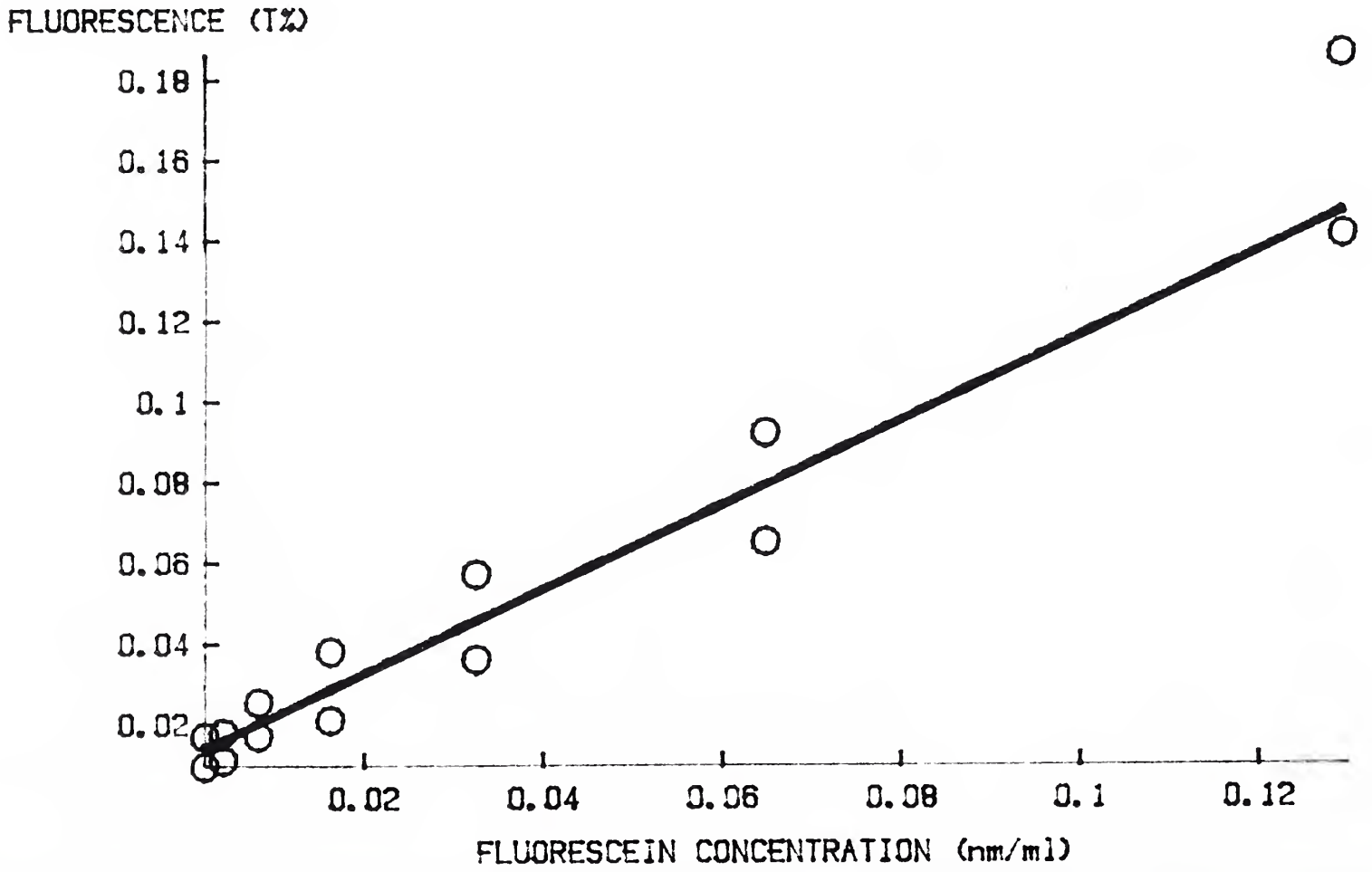


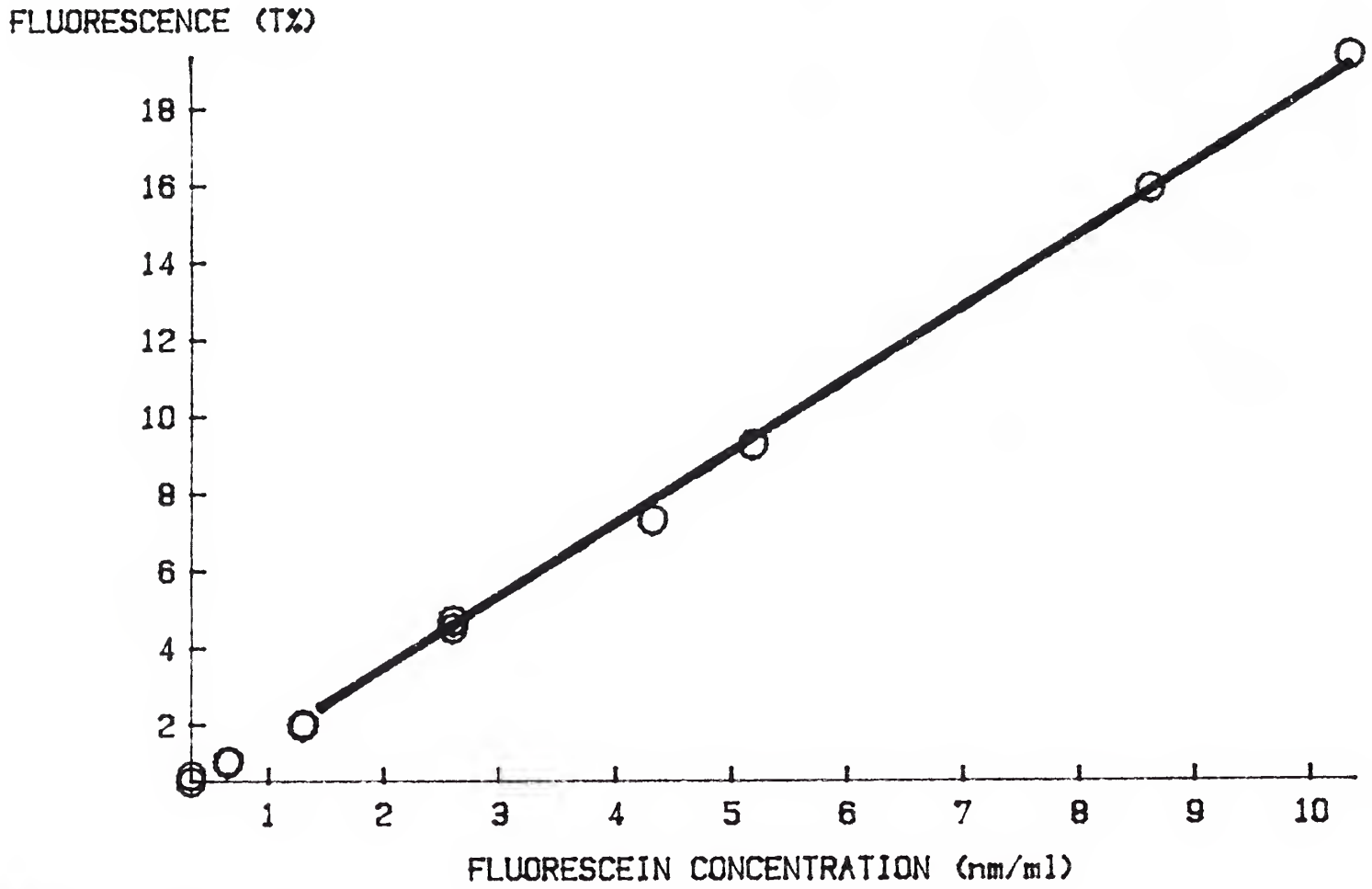
Figure 12

STANDARD CURVE
FLUORESCENCE VERSUS FLUORESCEIN CONCENTRATION



T% = 1.16 (nm/ml) − .0098
CORRELATION COEFFICIENT = .973

STANDARD CURVE
FLUORESCENCE VERSUS FLUORESCEIN CONCENTRATION



T% = 1.86 (nm/ml) - .247

CORRELATION COEFFICIENT = .999

solution concentrations used. Linear regression analysis provided equations for calculating the concentration of fluorescein and fluorescein-labelled dextran solutions from measured fluorescence.

In Vivo Experiments

Albino rabbits were anesthetized with 1 to 2 ml of 20 mg/ml xylazine mixed in a 1:1 ratio with 100 mg/ml ketamine HCl administered intramuscularly. Mydriasis was accomplished with phenylephrine HCl 2.5% (Neosynephrine) and tropicamide 1% (Mydriafair). Lens extraction was performed on the right eye of each rabbit. A partial thickness limbal incision was made with a No. 67 Beaver blade parallel to the superior limbus for 150 degrees. The cornea was penetrated at the center of the incision by a No. 75 Beaver blade and the anterior chamber was flushed with 0.1 ml of 1000 USP units/ml heparin in BSS. A cystotome consisting of a bent tipped 27 gauge needle was inserted into the anterior chamber and the anterior lens capsule widely incised. The corneal incision was extended with corneoscleral scissors and the lens nucleus expressed through the wound. The cortex was irrigated from the anterior chamber using a blunt 23 gauge needle. In four of the rabbits, a large surgical posterior capsulotomy was performed. The wound was closed with a running 10-0 nylon suture. Topical gentamicin sulfate and corticosteroid were applied three times during the first hour after the operation.

One to three months following lens extraction, the rabbits were premedicated with 526 mg of aspirin suppositories and anesthetized. Two 25 gauge needles were placed intracamerally in each eye by tracking 1 to 2 mm

through the corneal stroma before entering the anterior chamber. One of the needles in each eye led, via polyethylene tubing, to a reservoir of saline at a height equivalent to 20 mm Hg and the other to a Harvard perfusion pump. The fluorescein solution was initially infused at a rate of 10 ml/min. The infusing solution was at room temperature. After three hours of perfusion, the aqueous was aspirated using a 27 gauge needle into a pre-weighed tuberculin syringe. The weight and volume of the aqueous aspirate was recorded and fluorescence measured. The anterior chamber was quickly flushed with normal saline. The animals were sacrificed and the eyes rapidly enucleated. The globes were immediately frozen in liquid nitrogen.

The eyes were dissected while frozen. A Bard-Parker No. 15 blade was used to make a 360 degree perilimbal incision and to make four anteroposterior scleral incisions 90 degrees apart extending from the limbus to roughly 5 mm from the optic nerve. The anterior segment structures, including the lens in the phakic eyes, were removed as the sclera was peeled back. The frozen vitreous was transferred to a preweighed test tube. Samples of retina and choroid, optic nerve head, and sclera were also obtained and placed in weighed test tubes. Each tube was reweighed and the samples homogenized with 1 ml of normal saline. The tubes were placed in a centrifuge at 3650 rpm (2000 x gravity) for 15 minutes. The supernatant was transferred to a clear plastic cuvette and fluorescence measured. The fluorescein concentration in the original sample was then calculated.

Levels of baseline fluorescence in aqueous, vitreous, retinal and choroid, optic nerve head and sclera were measured in four rabbit eyes using an identical technique.

CALCULATIONS

The equation for determining the apparent solute permeability coefficient, w' is[53]

$$w' = \frac{Js}{(RTdC)} \qquad (1)$$

where Js, the solute flux is given by

$$Js = \frac{dNs/dt}{A} \qquad (2)$$

and dNs/dt is the rate of change in concentration of the solute on one side of the membrane in $mol/cm^3 sec$; A is the area of membrane available for diffusion in cm^2; R is a gas constant (8.31×10^7 erg/mol.^oK); T is the temperature in degrees Kelvin; and dC is the concentration gradient in mol/cm^3. The units of w', the apparent solute permeability coefficient, are therefore mol/sec.dyne.

The concentration gradient (dC) is the difference in solute concentration across the barrier. For the <u>in vitro</u> diffusion experiment, dC is the average difference in fluorescein or dextran concentration measured across the posterior lens capsule at one to two hour intervals. Because the rate of solute diffusion was small in relation

to the initial test solution concentration and both chambers were constantly stirred, the concentration gradient was constant during the experiment.

In the *in vivo* perfusion experiments the aqueous was stirred but not the vitreous. Accumulation of fluorescein in the anterior vitreous could reduce the effective concentration gradient in these eyes. Assuming that fluorescein accumulation occurred at a constant rate, dC was estimated by subtracting half of the final vitreous fluorescein concentration from the final aqueous concentration.

The rate of concentration change (dNs/dt) is the concentration of solute moving through the barrier per unit time. In the *in vitro* experiment this was calculate by linear regression of solute concentration in the chamber on the posteior side of the lens capsule versus time. The rate of concentration change was constant as evidenced by linear regression analysis.

RESULTS

In Vitro

Rabbit capsules [table 1] were significantly less permeable to 40,000 dalton dextran than to 18,900 dalton dextran ($p < .025$) or fluorescein sodium (MW = 376)($p < .001$). However, differences in capsule permeability among solutes of molecular weight less than 18,900 were not significant.

Human capsules [table 1] were significantly less permeable to 40,000 dalton dextran than to fluorescein sodium ($p = .026$). Human capsule permeability to 18,900 dalton dextran was similar to fluorescein sodium; however, the number of subjects in this group was too small for statistical comparison.

Human capsules were more permeable than rabbit capsules for 40,000 dalton dextran ($p < .005$). However, there were no significant differences between rabbit and human capsule permeability for dextrans of molecular weight equal to or less than 18,900. Greater individual variation in permeability was seen among human lens capsules than among albino rabbit capsules.

The relation between solute flux, Js, and fluorescein concentration was examined for the isolated rabbit posterior lens capsules [graph 1]. This relationship was linear with a correlation coefficient of .716 ($p = .014$).

TABLE 1

IN VITRO EXPERIMENTS

RABBIT CAPSULES

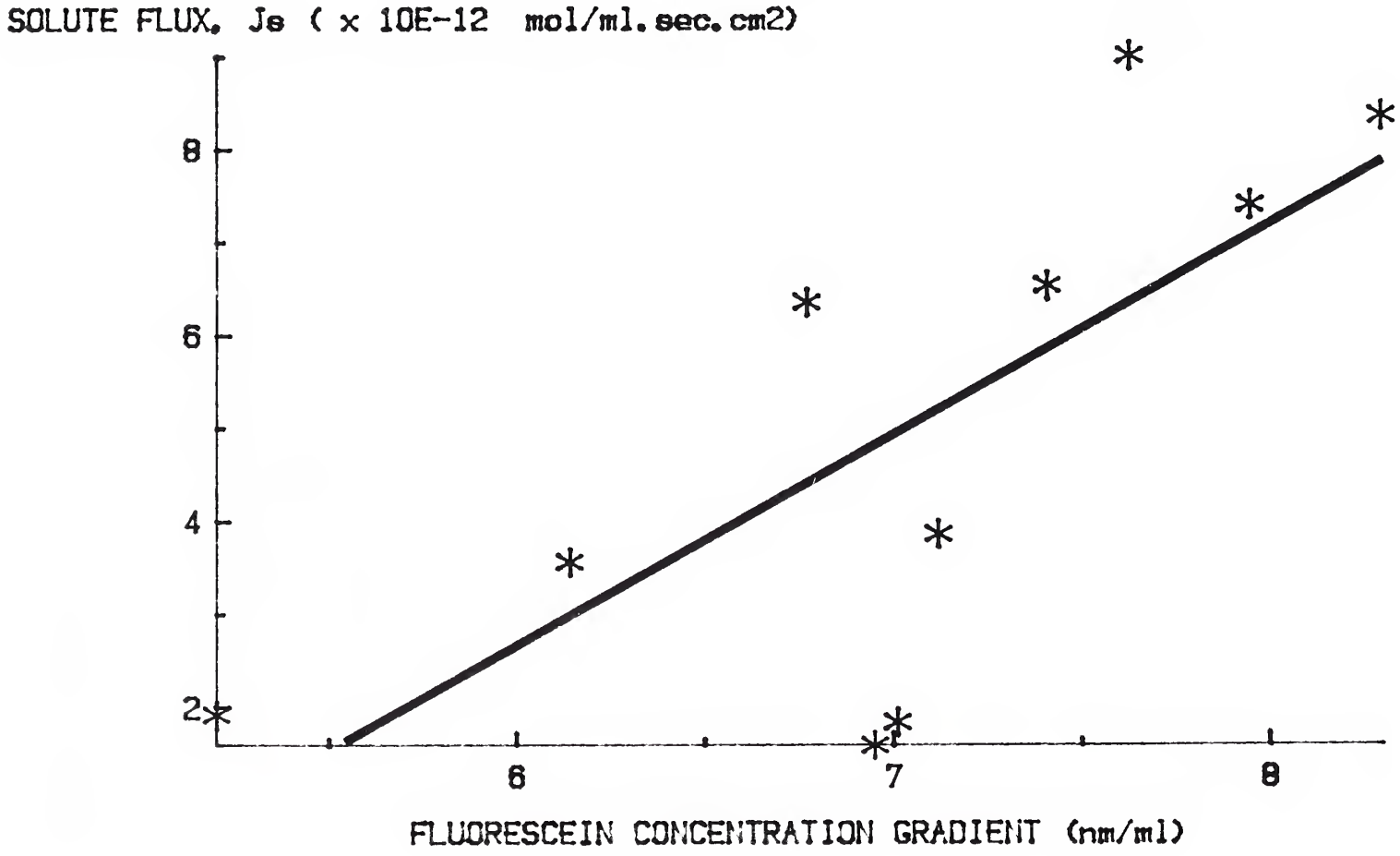
NO. OF CAPSULES	MOLECULAR WEIGHT (daltons)	APPARENT PERMEABILITY COEFFICIENT, $\underline{w}'$ (mol/sec.dyne)
8	376	$5.41 \pm .728 \times 10^{-16}$
5	4000	$6.35 \pm 4.41 \times 10^{-16}$
5	18900	$4.32 \pm 1.24 \times 10^{-16}$
4	40000	$7.61 \pm 1.58 \times 10^{-17}$

HUMAN CAPSULES

NO. OF CAPSULES	MOLECULAR WEIGHT (daltons)	APPARENT PERMEABILITY COEFFICIENT, $\underline{w}'$ (mol/sec.dyne)
4	376	$3.59 \pm 1.80 \times 10^{-16}$
1	18900	4.47×10^{-16}
4	40000	$1.65 \pm .165 \times 10^{-17}$

GRAPH 1

ALBINO RABBIT POSTERIOR LENS CAPSULES
IN VITRO
SOLUTE FLUX (Js) VERSUS CONCENTRATION GRADIENT



R = .716
Js = 2.27 (nm/ml) - 10.9

N = 10 CAPSULES

In Vivo: [table 2]

Mean apparent permeability coefficients for aphakic eyes with an intact posterior lens capsule (ECCE/CI), for aphakic eyes with a large posterior capsulotomy (ECCE/CO), and for contralateral phakic eyes belonging to each group (Phakic/CI) and (Phakic/CO) were calculated [table 3].

The mean permeability coefficient for aphakic eyes with a posterior capsulotomy (ECCE/CO) was 46 times greater than for aphakic eyes with an intact posterior lens capsule (ECCE/CI), although the variance was great. The mean apparent permeability coefficient for aphakic eyes with an intact posterior lens cpasule (ECCE/CI) was twice that of the contralateral phakic eyes in the same subjects (Phakic/CI). The ratio of mean coefficients for aphakic eyes with a posterior capsulotomy (ECCE/CO) to their contralateral phakic eyes (Phakic/CO) was 6.

Additional comparisons were made between the apparent permeability coefficient of fluorescein for isolated rabbit posterior lens capsules in vitro ($5.41 \pm .728 \times 10^{-16}$ mol/sec.dyne) and the values obtained by perfusion of aphakic eyes with ($1.60 \pm 1.02 \times 10^{-15}$ mol/sec.dyne = w'ECCE/CI) and without ($7.30 \pm 6.65 \times 10^{-14}$ mol/sec.dyne = w'ECCE/CO) an intact posterior lens capsule in vivo. The apparent permeability coefficient of perfused living aphakic eyes with an intact posterior lens capsule (ECCE/CI) was three times greater than the coefficient for the isolated

TABLE 2

IN VIVO EXPERIMENTS

APPARENT PERMEABILITY COEFFICIENT, w' (mol/sec.dyne)

CAPSULE INTACT GROUP

RABBIT	ECCE/CI(RE)	Phakic/CI(LE)	% DIFF.
I	4.55 X 10-15	3.00 X 10-15	+52
II	2.12 X 10-16	7.10 X 10-17	+199
III	2.19 X 10-16	2.05 X 10-17	+968
IV	1.42 X 10-15	1.61 X 10-16	+782

* % DIFF. = PERCENT DIFFERENCE = (w' ECCE/CI - w' Phakic/CI)/w' Phakic/CI x 100%.

CAPSULOTOMY GROUP

RABBIT	ECCE/CO(RE)	Phakic/CO(LE)	%DIFF.
V	1.91×10^{-14}	2.80×10^{-14}	-32
VI	4.16×10^{-16}	1.14×10^{-15}	-64
VII	2.72×10^{-13}	1.94×10^{-14}	+1302
VIII	4.93×10^{-16}	9.49×10^{-16}	-48

*%DIFF. = PERCENT DIFFERENCE = (w' ECCE/CO - w' Phakic/CO)/ w' Phakic/CO x 100 %.

In Vivo

TABLE 3

Apparent permeability coefficient by intracameral perfusion of rabbit eyes with fluorescein (MW =376.28) solution.

EYES	TREATMENT*	MEAN w' (mol/sec.dyne)	RATIO (RE:LE)
4 (RE)	ECCE/CI	$1.60 \pm 1.02 \times 10^{-15}$	
4 (LE)	Phakic/CI	$8.13 \pm 7.29 \times 10^{-16}$	2:1
4 (RE)	ECCE/CO	$7.30 \pm 6.65 \times 10^{-14}$	
4 (LE)	Phakic/CO	$1.24 \pm .677 \times 10^{-14}$	6:1

*ECCE/CI are aphakic eyes with an intact posterior lens capsule and Phakic/CI are contralateral phakic eyes in the same rabbits. ECCE/CO are aphakic eyes with a posterior capsulotomy and Phakic/CO are contraleteral phakic eyes in the same rabbits.

TABLE 4

Baseline Fluorescence in rabbit ocular tissue (nm of fluorescein-equivalents)

AQUEOUS	VITREOUS	RETINA+CHOROID	OPT N HEAD	SCLERA
0	.559	.455	.041	.041
0	.077	.131	.826	47.4
0	.550	.529	.883	
0	1.00	1.55	.826	

posterior lens capsule. This may represent fluorescein movement through the zonule; however, there was large variance in w' for ECCE/CI. In contrast, the apparent permeability coefficient for perfused aphakic eyes with a large posterior capsulotomy (ECCE/CO) exceeded the coefficient for the isolated posterior lens capsule by a factor of 135.

Variable levels of baseline fluorescence were detected in the ocular tissues [table 4]. In general, fluorescence was highest in vascularized tissues. Vitreous fluorescence ranged from .077 to 1.00 nM fluorescein-equivalent in the four eyes tested. Variation in baseline fluorescence may hav contributed to variance in the in vivo data.

DISCUSSION

The posterior lens capsule is a basement membrane secreted by embryonic lens epithelium[54]. It is composed of glycoprotein, with a 10 percent carbohydrate content[12,53,55]. Although the capsule contains enzymes, ATP, and glycolytic intermediates, it is not independently metabolically active54. The capsule is antigenically and chemically similar to the basement membranes of kidney glomeruli, spleen, lung, and blood vessels[54,56].

The lens capsule is a composite membrane. Its increasing thickness with growth is contributed to by the addition of layers[57]. The lens capsule is thinnest at the poles and equator. The posterior lens capsule is thickest in an area 1 mm from the posterior pole[58]. The following measurements were recorded by Salzmann[59] in 1912:

AGE(years)	THICKNESS(mm)		
	Post. Pole	Post. Max.	Equator
2.5	2	18-22	7
35	4	23	17
71	2.3	9	9

According to the above data, there is a decrease in posterior capsule thickness in later adulthood.

The permeability coefficient is a measurable quantity with complex molecular significance; it depends on the barrier's thickness, its composition, and on the diffusivity of the molecule through the barrier. Observation of the relative ability of solutes to cross the posterior lens capsule has been found by previous investigators to be a more accurate method of determining permeability than structural or chemical analysis of the barrier itself.

In 1930 Freidenwald conducted the first investigation and cross species comparison of posterior lens capsule permeability[60]. After noting that the pores in the lens capsule are submicroscopic and that the capsule is negatively charged relative to physiologic saline solution, he proceeded to measure permeability empirically using colloids, dyes, electrolytes, sugars and water[60]. The capsules were freely permeable to electrolytes and sugars with permeability decreasing as molecular size increased. As one might predict given the capsule's negative charge, it is slightly more permeable to electropositive than electronegative colloids and nearly impermeable to neutral proteins[60]. Animal capsules were contrasted on the basis of their selectivity for tetrachlorphenolsulphonpthalein and vital red. Considerable individual variability existed among adult animals but the differences were not a large as between species[60]. Adult animal capsules were much less permeable than capsules from young animals[60].

Francois and Rabaey[61] (1958) used microelectrophoresis on agar to test the permeability of the anterior ox lens capsule to lens proteins. The capsule was impermeable to alpha-crystalline (a high molecular weight protein) and to anionic, rapidly moving fractions. The capsule was highly permeable to the most basic fractions of medium molecular weight and to slow moving (less negatively charged), low molecular weight, embryonal protein fractions derived from the lens nucleus. This work demonstrates the importance of both molecular weight and polarity in determining a substance's ability to pass through the anterior lens capsule.

Fels (1971) tested the permeability of isolated rabbit, bovine, and human lens capsules to D- and L-tryptophan[53]. His interest in this question stemmed from studies by Ciusa and Barbiroli[62,63] in which l-amino acids were absorbed by the lens more rapidly than the corresponding d-isomers. This had been interpreted to represent selectivity on the part of the lens capsule for l-amino acids. Fels, however, found no significant difference in permeability between the two tryptophan isomers for any of the three species' lens capsules. Fels obtained an apparent permeability coefficient, w', of approximately 3×10^{-17} mol/sec.dyne for tryptophan (MW = 204 daltons)[53]. Whether anterior or posterior lens capsules were used was not specified.

Ozaki quantified <u>in vitro</u> permeability of posterior lens capsules from human cataractuous and rabbit lenses to

methylmethacrylate monomer (MW 100)[12], glucose (MW 180)[64], ^{3}H-prostaglandin E_1 (MW 354)[64], ^{3}H-prostaglandin F_2alpha (MW 354)[64], epinephrine (MW 193)[65], fluorescein sodium (MW 376)[12], and trypan blue (MW 961)[65]. Trypan blue had an apparent permeability coefficeint of w' = 10^{-17}mol/sec.dyne for the rabbit or human posterior lens capsules. Prostaglandin E_1 and methylmethacrylate monomer, both hydrophobic substances, had permeability coefficients of w' = 10^{-15}mol/sec.dyne. The hydrophilic substances, prostaglandin F_2alpha, fluorescein sodium, glucose and epinephrine had permeability coefficients of w' = 10^{-15}mol/sec.dyne*. The results of Ozaki's study indicate that the posterior lens capsule can function as a barrier to large non-electrolytes or negative electrolytes such as lipids, hyaluronic acid, and enzymes[65]. Molecular size and polarity in physiologic solutions were again important factors in determining the capsule's barrier function.

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* Permeability was expressed in Dr. Ozaki's paper as a transfer coefficient-apparent permeability constant, k, which is related to the apparent permeability coefficient, w', by[66]

$$k = w'RT \text{ at constant volume}$$

where R = 8.31 x 10^{7}erg/mol.^{o}K and T is the temperature in degrees Kelvin. The units of k are cm/sec. This conversion was made for presentation of the above results in this paper.

Thompson and Glaser investigated the role of extracapsular lens extraction with and without posterior capsulotomy on the movement of 20,000 and 70,000 dalton tritiated dextrans from rabbit vitreous to aqueous *in vivo*[67,68]. The aqueous concentration of 20,000 dalton dextran 3.5 hours after intravitreal injection was increased 14-fold after lensectomy with posterior capsulotomy and 10-fold after lensectomy with an intact posterior lens capsule. The rate of anterior movement of the 70,000 dalton dextran was increased fourfold in the eyes that had extracapsular lensectomy both with and without an intact posterior lens capsule. Thus, capsulotomy allowed increased movement of the 20,000 dalton dextran68. The abscence of a similar increase for the 70,000 dalton dextran may be related to a difference in sampling size or slowed diffusion of this larger molecule through the vitreous[68].

The formula for determining the apparent permeability coefficient was designed for an inert semipermeable composite membrane. The membrane's thickness, composition or homogeneity need not be specified. Calculations are based on the initial concentration gradient across the membrane, the rate of change of concentration gradient, the area of membrane available for diffusion, and the temperature. These equations permitted *in vitro* study of the isolated posterior lens capsule and *in vivo* study of the lens-zonular diaphragm, capsular-zonular diaphragm, and anterior hyaloid face in a comparable, quantitative manner.

The equation derived by Kedem and Katchalsky (1958)[53] for determining the apparent solute permeability coefficient, w' is as follows:

$$w' = Js/dPs$$

Where dPs is the osmotic pressure difference69 across the membrane given by

$$dPs = RTdC$$

and Js is the solute flux given by

$$Js = \frac{dNs/dt}{A}$$

Thus, the apparent permeability coefficient, w', is given by

$$w' = \frac{Js}{(RTdC)} \qquad Jv=0$$

The above formula applies only to experiments in which there is a constant solute flux (Js), or Jv=0[53,69]. In a non-facilitated diffusion system the solute flux is dependent on the concentration gradient. Hence, Jv=0 only when the concentration gradient is constant[56]. Constant stirring and a large initial concentration gradient

(>10nm/ml) compared to the amount of solute diffusing through the membrane (<0.1nm/ml) prevented significant fluctuations in concentration gradient during the in vitro experiments. In the in vivo experiments, lack of stirring in the vitreous compartment made it more difficult to insure a constant effective concentration gradient. Assuming that fluorescein accumulation in the anterior vitreous occurred at a constant rate, the effective concentration gradient was estimated by subtracting half of the final vitreous concentration of fluorescein from the final aqueous concentration. In addition, the perfusion was limited to three hours.

Even with constant stirring of the solutions on both sides of the membrane, an unstirred layer on the membrane's surface can interfere with accurate measurement of membrane permeability. Investigators have therefore preferred the term "apparent solute permeability coefficient, w'" to "solute permeability coefficient, w"[69]. The relation between w and w' is given by[69]

$$\frac{1}{w'} = \frac{1}{w} + \frac{2RTd}{D}$$

where D is the diffusion coefficient in solution and d is the thickness of the unstirred layer. However, for slowly penetrating solutes (eg. those with $w<10^{-15}$mol/sec.dyne), this calculation may be neglected[69]. In these experiments,

w' was small enough to be considered equivalent to the solute permeability coefficient, w.

The greater individual variability seen in the human *in vitro* data compared to the albino rabbit *in vitro* data may reflect greater homogeneity in a population of young, healthy albino rabbits. The human donor lens capsules were primarily from elderly individuals and neither the ocular nor the systemic histories of these eyes was available. In the case of human capsules, environmental, genetic, aging and disease processes may have influenced permeability. No significant differences were seen between the rabbit and human *in vitro* data except for the largest dextran.

Both human and rabbit data showed decreasing permeability for solutes of increasing molecular weight. This supports the model of the capsule as a semipermeable membrane with selectivity on the basis of molecular size.

Fluorescence in the vitreous of the perfused aphakic eyes with an intact posterior lens capsule exceeded that expected on the basis of the *in vitro* experiments. This difference is to large to be accounted for by the measured natural vitreous fluorescence. Hence, there appears to be fluorescein movement around the lens capsule, through the zonule.

The presence of an intact posterior lens capsule reduced the permeability coefficient by a factor of 46 in aphakic eyes. Thus, fluorescein movement through the zonule

is not sufficient to abolish the relative barrier effect of the posterior lens capsule.

The permeability coefficient for the capsular-zonular diaphragm was twice that of the lens-zonular diaphragm. This difference (8×10^{-16} mol/sec.dyne) is of similar magnitude to the permeability coefficient of the isolated posterior lens capsule (5.41×10^{-16} mol/sec.dyne) and may represent fluorescein movement through the capsule, uptake of fluorescein by the lens in the phakic eye, and/or partial disruption of the zonular ligament resulting from the lens extraction procedure.

Although the mean apparent permeability coefficient of aphakic perfused eyes with a posterior capsulotomy (ECCE/CO) was greater than the mean coefficient of contralateral phakic eyes (Phakic/CO), the reverse was true for several individual pairs of eyes [table 2]. This may represent back-diffusion of fluorescein into the aqueous through the capsulotomy. Clearly, whether fluorescein remains in the vitreous or returns to the aqueous in these eyes depends on a balance of forces including reistance of the anterior hyaloid face, and osmotic activity of fluorescein accumulating in the anterior vitreous as well as fluorescein in the anterior chamber. Nevertheless, when the difference between contralateral eyes was large, it was in the direction of greater apparent permeability in the aphakic eyes.

Individual variation in baseline fluorescence of the vitreous may have contributed both to the large statistical variance in the *in vivo* data and to the finding of higher *in vivo* apparent permeability coefficients compared to those for the isolated lens capsules. The large individual variability of this quantity made it impractical to use a correction factor in the *in vivo* experiments. Higher perfusion concentrations or the use of another tracer (ie. tritium) would have eliminated the influence of baseline fluorescence on experimental results and better duplicated the *in vitro* situation.

Methods used here in determining permeability apply only to accellular, inert semipermeable membranes. The posterior lens capsule has been described as a `coarse molecular screen' lacking the selectivity of cellular membranes[53,60]. The solute flux (Js) or rate of concentration change per unit area of an inert semipermeable membrane is linearly related to concentration gradient[70]. In contrast, facilitated diffusion systems (ie. cellular membranes) are characterized by diffusion curves which plateau and are described by a different kinetic model[60,71]. In these systems the solute flux is constant for concentration gradients above a critical value representing saturation of the transport mechanism[53].

The capsule is constructed in staggered overlapping layers giving it an indeterminate surface area with a variety of adsorbed, unstirred fluid layers[53]. The

possibility of the lens capsule's heterogeneous lamellar structure interfering with its ability to conform to linear phenomenologic equations has been considered both here and in previous investigations[53,70]. Fels (1971) compared the solute flux for animal capsules to that of Visking tubing, an inert homogeneous man-made membrane[53]. He found the solute flux to be linear for both with a latent period preceeding linear flow in the case of human capsules[53]. The relation between solute flux, Js, and fluorescein concentration was linear for in vitro rabbit capsule data [graph 1]. Hence, no evidence of facilitated or anomalous diffusion has been observed in this or previous studies with the posterior lens capsule[12,60,61,64,65].

In agreement with the empirical findings of Freidenwald[60], Francois[61], Ozaki[64,65], and Thompson[68] there was decreasing permeability with increasing molecular size. However, in contrast to Freidenwald's in vitro results[60], permeabilities of rabbit and human capsules were similar except for the largest, 40,000 dalton dextran for which human capsules were more permeable. Also, the intra-species variability in capsule permeability noted by Freidenwald[60] was reproduced for the human data but not for the albino rabbit data. This last difference may reflect greater homogeneity of the subject population consisting only of albino rabbits of approximately the same age.

The value obtained by Fels[53] for lens capsule permeability to tryptophan ($w'=3 \times 10^{-17}$ mol/sec.dyne[53])

approximates the permeability of the highest molecular weight dextran (40,000 dalton) tested here. Tryptophan, a 204 dalton molecular weight amino acid, has a non-polar R group or side chain containing an aromatic ring[72]. Its hydrophobic nature may explain the lens capsule's low permeability for this amino acid.

Ozaki's apparent permeability coefficient of the posterior lens capsule to fluorescein (10^{-15}mol/sec.dyne)[64] was greater than the one measured here (10^{-16}mol/sec.dyne). The principle differences in experimental technique between the two studies are the use of a shorter incubation time of one hour and the use of artificial aqueous humor as a solvent by Ozaki. The effect of these difference on apparent permeability coefficient is not entirely clear; however, the shorter incubation time may have minimized the effect of an unstirred layer on the posterior surface of the lens capsule on effective concentration gradient. As the unstirred layer accumulates fluorescein it can artifactitiously diminish concentration gradient and thus permeability coefficient.

Using intravitreal injections of 20,000 and 70,000 dalton dextrans, Thompson and Glaser found greater aqueous levels of tracer in aphakic eyes with an intact posterior lens capsule compared to phakic eyes[67,68]. Similarly, there was more movement of fluorescein from aqueous into vitreous across the capsular-zonular diaphragm than across the lens-zonular diaphragm in the study performed here. Both of

these observations may represent tracer movement through the lens capsule, or through a partially disrupted zonule in the aphakic eye, or uptake of tracer by the lens in the phakic eye. Tracer movement across the len-zonular diaphragm in both Thompson and Glaser's experiments and in the in vivo fluorescein perfusion experiment reported here suggests movement of tacer through the zonule. These investigators also found a greater increase in anterior movement of 20,000 dalton dextran in aphakic eyes with a posterior capsulotomy compared to aphakic eyes with an intact posterior lens capsule[67,68]. This is consistent with the in vivo perfusion data indicating the posterior lens capsule to be a relative barrier to the diffusion of tracers. The abscence of an observed difference in anterior diffusion of the 70,000 dalton dextran among eyes with and without and intact posterior lens capsule may be secondary to slowed movement through the vitreous and may point out an advantage to studying posterior movement of smaller tracers from aqueous to vitreous.

As aphakia becomes more common in the population, the potential barrier functions of the posterior lens capsule, capsular-zonular diaphragm, lens-zonular diaphragm, and anterior hyaloid face become important. An intact posterior lens capsule may reduce access of topical drugs, infectious agents[52], and aqueous substances[12,14] to the retinal and vitreous. The capsular-zonular diaphragm may also limit the

access of vitreous[51,68], retina and retinal pigment epithelium derived substances to the anterior segment.

By reducing diffusion of prostaglandins released into the aqueous to the vitreous, the capsular- zonular diaphragm may reduce the eye's susceptibility to aphakic cystoid macular edema[12,14]. Fluorescein and prostaglandins are of approximately equal molecular weight. In addition, they are both carboxylic acids and exist predominately in anionic form at physiologic pH. The data presented here show the capsular-zonular diaphragm to be a relative but not absolute barrier to fluorescein. Although the capsular-zonular diaphragm wa a less effective barrier to fluorescein than the lens-zonular diaphragm, it was more effective than the anterior vitreous face. The barrier effect of the capsule and surrounding zonule to prostaglandins may combine with physiologic mehanisms of aqueous clearance and prostaglandin deactivation to reduce access of these substances to the retina. This may explain the apparent relative protection provided by the intact posterior lens capsule against cystoid macular edema in aphakic patients[12,14].

Lensectomy increases the incidence of iris neovascularization in patients undergoing vitrectomy for complications of diabetic retinopathy[41,42]. This may represent removal of vitreous inhibitors of vasoproliferation[46] and loss of physical barriers to anterior movement of retina[41,44] and retinal pigment epithelium[47] derived stimulators of neovascularization. A

potential angiogenic factor isolated from bovine retina has been characterized as an anion of greater than 50,000 molecular weight[41,48,49,50]. Combining the data collected here with previous studies on posterior lens capsule permeability, the capsular-zonular diaphragm can reduce anterior movement of an anion this size. Further studies regarding the influence of the lens, zonule and lens capsule on movement of angiogenic factors in the eye may clarify their etiologic role in iris rubeosis and enhance the clinical approach to diabetic patients needing vitrectomy and lens extraction.

The method of measuring permeability employed in this study is empirical. An attempt was made to obtain <u>in vitro</u> and <u>in vivo</u> data in a form permitting quantitative comparison. In the future, this approach may be useful in testing capsule permeability to other substances of interest in eye research. These substances may include endogenous inflammatory agents (ie. prostaglandins, leukotrienes, substance P, ATP, epinephrine), experimental angiogenic factors, topical and intracamerally administered pharmacologic agents, and pathogens (ie. viruses, bacteria, fungi). The experimental technique is also applicable to testing permeability of any other acellular, composite membrane.

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